Enhanced dopamine-dependent hippocampal plasticity after single MK-801 application

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Abstract

Dopaminergic hyperfunction and N-methyl-D-aspartate receptor (NMDAR) hypofunction have both been implicated in psychosis. Dopamine-releasing drugs and NMDAR antagonists replicate symptoms associated with psychosis in healthy humans and exacerbate symptoms in patients with schizophrenia. Though hippocampal dysfunction contributes to psychosis, the impact of NMDAR hypofunction on hippocampal plasticity remains poorly understood. Here, we used an NMDAR antagonist rodent model of psychosis to investigate hippocampal long-term potentiation (LTP). We found that single systemic NMDAR antagonism results in a region-specific, presynaptic LTP at hippocampal CA1-subiculum synapses that is induced by activation of D1/D5 dopamine receptors and modulated by L-type voltage-gated Ca^{2+} channels. Thereby, our findings may provide a cellular mechanism how NMDAR antagonism can lead to an enhanced hippocampal output causing activation of the hippocampus-ventral tegmental area-loop and overdrive of the dopamine system.

Introduction

Pathophysiological concepts of psychosis are based on dysregulated dopaminergic neurotransmission due to two fundamental observations: First, stimulants which enhance dopaminergic transmission (e.g. cocaine or amphetamines) can lead to psychosis resembling positive symptoms of schizophrenia (Lieberman *et al*, 1987). Second, currently used antipsychotics block dopamine (DA) receptors (Creese *et al*, 1976). In contrast to dopaminergic agents, antagonists of the glutamatergic NMDA receptor (NMDAR) like phencyclidine, ketamine and MK-801 can replicate not only positive, but also negative and cognitive symptoms associated with psychosis in healthy humans. Moreover, they exacerbate symptoms in patients with schizophrenia (Luby *et al*, 1959). Consequently, psychosis was linked to NMDAR hypofunction (Javitt and Zukin, 1991; Olney *et al*, 1999) and contemporary models of psychosis stress interactions between the glutamatergic and the dopaminergic neurotransmitter systems (Lisman *et al*, 2008). NMDAR antagonists can increase the release of DA in various cortical and subcortical brain regions (Breier *et al*, 1998; Deutch *et al*, 1987; Whitton *et al*, 1992). Dopaminergic fibers arising from the ventral tegmental area (VTA) form a functional loop with the hippocampus, in particular with the CA1 and the subiculum (SUB) (Verney *et al*, 1985). Both hippocampal regions project to the nucleus accumbens, which regulates the release of DA in the hippocampus by disinhibition of the VTA (Brudzynski and Gibson, 1997; Floresco *et al*, 2001). Interestingly, experimental findings suggest a close functional relationship between memory formation in the hippocampus and dopaminergic neuromodulation (Lisman and Grace, 2005). Activation of D1/D5 DA receptors (D1/D5R) lowers the threshold for the induction of long-term potentiation (LTP), a cellular correlate for

memory formation, in CA1 and the SUB both in vitro and in vivo (Li *et al*, 2003; Otmakhova and Lisman, 1996; Roggenhofer *et al*, 2010, 2013). Aside from transient immediate psychosis-like effects, acute treatment with the NMDAR antagonist MK-801 induces lasting effects on hippocampal function and synaptic plasticity (Wiescholleck and Manahan-Vaughan, 2013). In fact, dysregulation in the interplay of NMDAR function and dopaminergic neuromodulation may underlie hippocampus-dependent deficits. Hence, single NMDAR antagonist administration in rodents represents a feasible approach to characterize the impact of temporary systemic hypoglutamatergic and hyperdopaminergic states on LTP in the hippocampus. Here, we report that after systemic NMDAR antagonism and in the drug-withdrawn state, CA1-SUB synapses show a region-specific, presynaptic LTP that is induced by D1/D5R. Thus, our results link the concepts of NMDAR hypofunction and dopaminergic hyperfunction, and potentially provide a cellular mechanism for hippocampal dysfunction after acute psychotic states.

Materials and Methods

Please refer to Supplementary Materials and Methods for additional information.

Animals: All procedures were performed in accordance to national and international guidelines and approved by local authorities. Male Wistar rats (4-6 weeks old) received a single intraperitoneal injection with either MK-801 ((5S,10R)-(+)-5-methyl-10,11-dihydro-5H-dibenzo(a,d)cyclohepten-5,10-imine maleate, 5 mg/kg body weight, except for the experiment shown in Fig. S1b: 0.5 mg/kg, dissolved in 0.9% saline) or 0.9% saline (10 ml/kg body weight).

Electrophysiology: Single-cell recordings were performed in horizontal hippocampal slices prepared as described (Wozny *et al*, 2008a, 2008b). To study synaptic plasticity at glutamatergic synapses, all experiments were performed in the presence of bicuculline (5 µM) to block $GABA_A$ receptor-mediated responses. Drugs were bath-applied except for BAPTA (30 mM in the intracellular solution). Afferent stimulation (Fig. S1a) was adjusted to give comparably sized evoked excitatory postsynaptic potentials/currents (EPSP/EPSC, ~30% of maximal amplitude, Table S1). EPSP/EPSCs were normalized to baseline values. LTP was calculated by averaging the responses collected during the last 5 min of each experiment. Miniature EPSCs (mEPSC) were recorded in the presence of tetrodotoxin (1 µM) and bicuculline (10 µM).

Histology: Postfixed slices containing a biocytin-labeled subicular pyramidal cell were immunohistochemically stained against D1R, D5R and vGluT1 and assessed by confocal microscopy.

Quantification of subicular DA levels: DA was measured by high performance liquid chromatography (HPLC) with electrochemical detection after extraction to alumina as previously described (Felice *et al*, 1978).

Ca^{2+} imaging: Imaging of stimulus-induced (20 pulses at 20 Hz) changes in Ca^{2+} fluorescence in the subicular field was performed in acute slices stained with the AM-ester form of Oregon Green 488 BAPTA-1 under submerged conditions. In order to dissect presynaptic Ca^{2+} signals, postsynaptic glutamatergic and GABAergic transmission was blocked with D-AP5 (50 µM), CNQX (30 µM), (RS)-MCPG (150 µM), bicuculline (10 µM) and CGP 55845 (20 µM) (Fig. S7). D1/D5Rs were activated by the agonist SKF38393 (100 µM) alone or in combination with nifedipine (20 µM). Changes in fluorescence over time in the subicular field were plotted as $\Delta F/F_0$ with F_0 representing the average fluorescence before stimulus onset. For analysis of stimulus-induced changes in presynaptic calcium fluorescence, the peak $\Delta F/F_0$ in regions of interest, excluding cell bodies of neurons and glia, was calculated.

Statistical analysis: Values are given as mean±SEM unless otherwise stated. Comparisons between two groups were analyzed by appropriate *t*-tests, nonparametric equivalents or Kolmogorov-Smirnov test. Multigroup comparisons were analyzed with one-way ANOVA or Kruskal-Wallis H-test followed by Dunnett's or Dunn's *post-hoc* test.

Results

LTP following systemic NMDAR antagonism

We first tested for LTP at Schaffer collateral (SC)-CA1 and CA1-SUB synapses using strong high-frequency stimulation (HFS, 4x 100 pulses at 100 Hz, 10 s inter-train interval). In line with previous findings (Wöhrl *et al*, 2007), LTP at SC-CA1 synapses was impaired in MK-801-treated rats 24 hours post injection (control: 1.69±0.11, n=8, p<0.001; MK-801: 1.11±0.11, n=8, Fig. 1a, c). In sharp contrast, LTP could be readily induced at CA1-SUB synapses (control: 2.23±0.30, n=11, p<0.01; MK-801: 2.58±0.49, n=9, p<0.05, Fig. 1d, f). Since strong HFS protocols can mask an altered threshold to induce LTP, we dampened the stimulation protocol to 10 pulses at 40 Hz. This attenuated HFS protocol failed to induce plasticity in CA1 and subicular pyramidal cells in control rats (CA1: 1.15±0.09, n=7, SUB: 1.08±0.07, n=10, Fig. 1b, c, e, f). In MK-801 treated rats, however, it resulted in a robust late-onset LTP exclusively in the SUB (CA1: 1.10±0.07, n=7, SUB: 2.73±0.36, n=9, p<0.001, Fig. 1b, c, e, f). Comparing injection of 0.5 mg/kg and 5 mg/kg of MK-801 showed no significant difference in LTP (0.5 mg: 2.12±0.38, n=7, p<0.05; 5 mg: 2.73±0.36, n=9, p<0.001, Fig. S1b). We also tested this attenuated stimulation protocol in granule cells of the dentate gyrus (DG) and CA3 pyramidal cells. Like in the CA1, HFS failed to induce LTP in the DG and CA3 (control: DG: 1.14±0.18, n=6, CA3: 1.34±0.19, n=5; MK-801: DG: 1.12±0.12, n=8, CA3: 1.38±0.19, n=5, Fig. S1d-f). Thus, we conclude that this form of LTP is unique to subicular neurons.

To identify how fast this subicular LTP appears following MK-801 injection and whether changes in plasticity are permanent, we also investigated LTP induction one hour, one and two weeks after systemic application of MK-801. Stable LTP could be

induced one hour after MK-801 treatment and one week but not two weeks after application (one hour: 2.31±0.49, n=6, p<0.05; one week: 2.24±0.40, n=8, p<0.05; two weeks: 1.08±0.09, n=13, Fig. S1c).

At various synapses in the central nervous system, LTP depends on NMDAR activation (Nicoll and Malenka, 1999). In the presence of the NMDAR antagonist AP5, HFS resulted in a different time course of LTP lacking the initial enhancement (2.20±0.22, n=9 vs. 1.46±0.07, n=7, p<0.05 at min 10-15, Fig. 2a). Yet, late-onset LTP still emerged in subicular pyramidal cells from MK-801-treated animals (3.43±0.53, n=7, p<0.01 at min 35-40, Fig. 2a). This suggests that this form of late-onset LTP consist of an NMDAR-dependent and an NMDAR-independent component.

NMDAR-independent LTP has been reported to depend on the activation of metabotropic glutamate receptors (mGluR) (O'Leary and O'Connor, 1999). But, MCPG, an antagonist of mGluR I/II, did not prevent LTP in cells from MK-801-treated rats (2.51±0.56, n=8, p<0.05, Fig. S2).

LTP in MK-801-treated rats depends on the activation of D1/D5Rs

Next, we tested if the LTP in MK-801-treated rats is mediated by endogenous DA released upon HFS. Using HPLC, we detected endogenous DA concentration in subicular tissue samples in both animal groups (control: 23.99±4.72 pg/mg wet tissue, n=8; MK-801-treated animals: 20.65±2.29 pg/mg wet tissue, n=8, Fig. S3). When D2 dopamine receptors (D2R), targeted by many commonly used antipsychotics, were blocked with the antagonist sulpiride, LTP could still be induced (2.11±0.21, n=9, p<0.001, Fig. 2b). However, the specific D1/D5R antagonist

SCH23390 blocked LTP (0.98±0.10, *n*=8, Fig. 2c). Baseline EPSP amplitudes were not affected by D1/D5R blockade (1.06±0.09, *n*=6, Fig. 2e). Up to this point it remained unclear whether activation of D1/D5Rs is mandatory for the induction or expression of this late-onset LTP. Therefore, we blocked D1/D5Rs with SCH23390 after applying electrical HFS. Under these conditions, we still observed a robust LTP concluding that D1/D5R activation is crucial for the induction, but not the expression of this form of LTP (2.61±0.61, *n*=7, $p<0.05$, Fig. 2d). Analysis of synaptic responses during 40 Hz stimulation revealed a more pronounced short-term depression when D1/D5Rs were blocked (Fig. 2f, g, Fig. S6). This result suggests that under conditions of sustained synaptic activity, rapid replenishment of the readily releasable pool of synaptic vesicle seems to be facilitated by tonic D1/D5R activation in MK-801-treated rats.

If D1/D5R activation is mandatory for the LTP in MK-801-treated rats, activating these receptors by a specific agonist should also result in LTP. Accordingly, bath-application of the specific D1/D5R agonist SKF38393 at various concentrations enhanced synaptic transmission in MK-801-treated rats (10 µM: 1.44±0.12, *n*=8, $p<0.05$; 30 µM: 1.39±0.10, *n*=7, $p<0.01$, Fig. 2h), but not in control slices (10 µM: 1.00±0.07, *n*=6, 30 µM: 1.04±0.08, *n*=9, Fig. 2h). To determine the expression site of this D1/D5R-mediated chemical enhancement of synaptic transmission in MK-801-treated animals, we analyzed the paired-pulse index (PPI). The PPI investigates the ability of synapses to increase transmitter release upon the second of two closely spaced afferent stimuli, and depends on residual Ca^{2+} levels in the presynaptic terminal (Zucker and Regehr, 2002). The PPI decreased after D1/D5R activation consistent with a presynaptic expression mechanism of this enhanced synaptic

transmission in MK-801-treated rats (10 µM: from 1.50±0.17 to 1.26±0.13, n=8, p<0.05, 30 µM: from 1.65±0.18 to 1.21±0.09, n=7, p<0.05, Fig. 2i).

LTP in MK-801-treated rats is expressed presynaptically and is independent of postsynaptic Ca^{2+}

We used different approaches to determine the site of HFS-induced LTP in MK-801-treated animals. The PPI decreased from 2.33±0.29 to 1.74±0.18 after LTP induction consistent with a presynaptic expression mechanism (n=9, p<0.05, Fig. 3a).

Additionally, we studied failure rates of minimal stimulation. LTP expression reduced failure rates from 71±4% to 30±9% (n=9, p<0.01, Fig. 3b), indicating an increase in transmitter release probability.

Changes in the coefficient of variation (CV)-index that accompany alterations in synaptic efficacy are likewise used to differentiate between pre- and postsynaptic mechanisms (Faber and Korn, 1991). Supporting a presynaptic mechanism, LTP induction went along with changes in the CV-index (baseline: 0.058±0.017, LTP: 0.023±0.007, n=9, p<0.05, Fig. S4).

To determine whether postsynaptic Ca^{2+} signaling is required for the LTP in MK-801-treated rats, we performed whole-cell patch-clamp recordings to ensure proper dialysis of cells with a Ca^{2+} buffer. We first reproduced the LTP in MK-801-treated animals under patch-clamp conditions and therefore adjusted the HFS to 200 pulses at 50 Hz applied in current-clamp mode. This resulted in a robust LTP in MK-801-treated rats (1.41±0.06, n=7, p<0.001, Fig. 3c), but only brief post-tetanic potentiation and no LTP in control animals (1.00±0.08, n=8, Fig. 3c). The magnitude of LTP in whole-cell patch-clamp recordings was smaller than in sharp microelectrode recordings, a fact that might be attributed to a difference in oxygen

supply under submerged and interface recording conditions (Hájos *et al*, 2009). Postsynaptic Ca^{2+} buffering with the fast Ca^{2+} chelator BAPTA did not prevent LTP induction in MK-801-treated rats (1.29±0.06, n=8, p<0.01, Fig. 3c), suggesting that LTP is independent of postsynaptic Ca^{2+} signaling.

D1/D5Rs modulate release probability of glutamate in MK-801-treated rats

Confocal imaging of biocytin-filled (postsynaptic marker) subicular bursting neurons in slices that were immunohistochemically labeled for vGluT1 (presynaptic marker) and D1R or D5R suggested the existence of pre- and postsynaptic D1Rs and D5Rs (Fig. 4a, b). All DA receptors are coupled to G-proteins. In general, activation of G-protein signaling pathways has been shown to modulate action potential-independent mEPSCs directly (Scanziani *et al*, 1992). Given a tonic basal activation of D1/D5Rs by endogenous DA, functional presynaptic D1/D5R could modulate transmitter release independent of action potential-triggered calcium influx (Bouron and Reuter, 1999). Therefore, we measured mEPSCs before and after the application of the specific D1/D5R antagonist SCH23390.

Only in MK-801-treated rats, D1/D5R blockade reduced mEPSC frequency without affecting the amplitudes (mEPSC frequency: control: 0.85±0.17 Hz during baseline, 0.83±0.13 Hz during SCH23390; MK-801: 0.99±0.19 Hz during baseline, 0.61±0.11 Hz during SCH23390, p<0.05; mEPSC amplitude: control: 16.12±0.99 pA during baseline, 16.28±0.94 pA during SCH23390; MK-801: 16.55±1.36 pA during baseline, 15.98±1.56 pA during SCH23390, n=8 in both animal groups, Fig. 4c-f). These results support the existence of functional D1/D5Rs located at the presynaptic site of the CA1-SUB synapse and indicate that activation of D1/D5Rs by endogenous DA tonically maintains glutamate release at CA1-SUB synapses in MK-801-treated rats.

LTP in MK-801-treated rats is mediated by the adenylate cyclase (AC)-cAMP-PKA signaling cascade

D1/D5R are positively coupled to the AC-cAMP-PKA signaling cascade (Missale *et al*, 1998). We studied whether blocking this pathway prevents the LTP in MK-801-treated animals. Indeed, preincubation of slices with the PKA inhibitor Rp-8-CPT-cAMPS blocked the induction of LTP in MK-801-treated animals (1.19±0.14, n=6, Fig. 5a). To test for differences in pathway function downstream of the D1/D5R between control and MK-801-treated rats, we activated the AC-cAMP-PKA cascade with forskolin. In line with a cAMP-mediated form of LTP, forskolin enhanced EPSC amplitudes. As we did not find differences between both animal groups (control: 1.62±0.20, n=6, p<0.05; MK-801: 1.63±0.20, n=6, p<0.05, Fig. 5b) alterations upstream of the AC-cAMP-PKA cascade have to be considered.

LTP in MK-801-treated rats is modulated by L-type voltage-gated Ca^{2+} channels

DA receptor activation can modulate L-type voltage-gated Ca^{2+} channel (LTCC) function via PKA-dependent phosphorylation of the α1 subunit of LTCCs and a subsequent increase of Ca^{2+} currents (Missale *et al*, 1998; Surmeier *et al*, 1995). Blocking LTCCs with nifedipine did not affect baseline EPSP amplitudes (1.01±0.09, n=7, Fig. S5a) but caused a substantial attenuation of LTP in MK-801-treated rats (1.49±0.08, n=9, p<0.001, Fig. 5c). Notably, applying nifedipine after HFS likewise attenuated LTP (1.52±0.16, n=9, p<0.05, Fig. 5d). Bath-application of diltiazem, a structurally different inhibitor of LTCC, also attenuated LTP (1.23±0.09, n=5, p<0.05, Fig. S5b). Thus, LTCCs seem to contribute to LTP expression in MK-801-treated

rats. Noteworthy, in control animals, NMDAR-dependent subicular LTP is not LTCC-dependent (1.98±0.30, n=5, p<0.05, Fig. S5c).

To test for altered presynaptic LTCC function in MK-801-treated animals, we recorded stimulus-induced changes in Ca^{2+} fluorescence signals in the subicular field in slices loaded with the Ca^{2+} indicator Oregon Green BAPTA-1. Stimulus-induced presynaptic Ca^{2+} transients were studied during pharmacological blockade of glutamatergic and GABAergic transmission (Liotta *et al*, 2012, Fig. S7). Activation of D1/D5Rs with the agonist SKF38393 resulted in a marked increase of stimulus-induced Ca^{2+} transients exclusively in MK-801-treated rats and this was prevented by co-application of the LTCC blocker nifedipine (control+SKF38393: 99.82±1.15% of baseline, n=6; MK-801+SKF38393: 108.77±3.48% of baseline, n=9, p<0.05; MK-801+SKF38393+nifedipine: 102.12±4.86% of baseline, n=6, Fig. 5e). These results indicate that LTCCs contribute to the D1/D5R-induced increase in presynaptic Ca^{2+} levels in MK-801-treated animals.

Discussion

The present study aimed to investigate LTP of hippocampal output structures after systemic NMDAR antagonism by single MK-801 administration. In control tissue, subicular LTP is NMDAR-dependent (Aoto *et al*, 2013; Wozny *et al*, 2008a, 2008b). In MK-801-treated rats, we found a presynaptic late-onset LTP restricted to subicular pyramidal cells. This LTP requires activation of D1/D5Rs, but not D2Rs, and the AC-cAMP-PKA cascade. It is modulated by LTCCs and independent of postsynaptic Ca^{2+} signaling (for summary see Fig. 5f). Though the expression of LTP (>5 min after stimulation) is NMDAR-independent, the initial phase (<5 min after stimulation) was

dependent on the (co-)activation of NMDAR and D1/D5R. Interestingly, the persistence of protein synthesis-dependent late-LTP (beyond 4 hours post induction) at SC-CA1 synapses requires the co-activation of NMDAR and D1/D5R (Navakkode *et al*, 2007; O'Carroll and Morris, 2004). However, we only investigated early-LTP (30 min post induction). Evidently, at least during this phase of LTP, there are alternative NMDAR-independent pathways that can lead to the D1/D5R-induced LTP at CA1-SUB synapses in MK-801-treated rats.

Several lines of evidence support a presynaptic expression of this LTP. LTP was accompanied by changes in PPI, transmission failure rates and the CV indicating an increase in presynaptic transmitter release. LTP was independent of postsynaptic Ca^{2+} and Ca^{2+} fluorescence recordings demonstrated a LTCC-modulated rise in Ca^{2+} signals after D1/D5R activation.

As we found no difference in the extent of forskolin-induced enhancement of EPSCs between control and MK-801 animals, alterations upstream of the AC-cAMP-PKA cascade or AC-independent mechanisms have to be considered. G-protein-mediated signaling can modulate transmitter release by regulating presynaptic Ca^{2+} influx and cAMP levels. A priming effect of cAMP in concert with Ca^{2+} has been suggested to interfere with the recruitment of synaptic vesicles during repetitive activity (Yao and Sakaba, 2010). This mechanism is compatible with the assumption that G-protein-coupled D1/D5Rs can regulate the efficacy of synaptic vesicle exocytosis. It might be crucial in preventing depletion of presynaptic vesicular glutamate stores under conditions of sustained activity, e.g. repetitive HFS during LTP induction.

In MK-801-treated animals, LTCCs contribute to the increased Ca^{2+} levels upon D1/D5R stimulation and D1/D5R-induced LTP expression. This could be due to increased LTCC expression, changes in subcellular localization or enhanced

functional properties of pre-existing LTCCs. Given that D1/D5Rs are positively coupled to the AC-cAMP-PKA pathway, an enhanced LTCC efficacy following D1/D5R activation by a PKA-dependent mechanism seems reasonable (Fourcaudot *et al*, 2008). Functional channel modification by proteins interacting with LTCCs like presynaptic active zone components should also be considered (Fourcaudot *et al*, 2008). LTCCs are known to play a crucial role in controlling neuronal excitability, synaptic plasticity and gene expression throughout the nervous system (Calin-Jageman and Lee, 2008). For example, a LTCC-dependent increase in glutamate release underlies the presynaptic LTP of cortical inputs to the lateral amygdala (Fourcaudot *et al*, 2009). The brain-specific pore-forming α_{1C} subunit of the LTCC, $Ca_V1.2$, is expressed pre- and postsynaptically in the hippocampus (Tippens *et al*, 2008). Mice with a hippocampus-specific knockout of *Cacna1c* encoding for $Ca_V1.2$ show a selective loss of protein synthesis-dependent, NMDAR-independent SC-CA1 late-phase LTP (Moosmang *et al*, 2005). Interestingly, recent clinical genetic association studies have established an association of polymorphisms in the α_{1C} subunit of the LTCC gene *(CACNA1C)* and schizophrenia (Bhat *et al*, 2012). Thus, LTCCs may link D1/D5R function and the altered expression of LTP after psychotic states. In clinical trials, LTCC blockers led to conflicting results, though cognition improving effects were reported in schizophrenic patients and ketamine-induced psychosis (Hollister and Trevino, 1999; Krupitsky *et al*, 2001). Of course, other substances and pathways like the therapeutic merits of the nitric oxide donor sodium nitroprusside should also be recognized as it has been shown to block the phencyclidine-induced activation of c-fos (Bujas-Bobanovic *et al*, 2000; Hallak *et al*, 2013).

Hippocampal dysfunction in and after psychosis has been suggested to include subfield-specific alterations in synaptic plasticity (Tamminga *et al*, 2012; Wieschollеck and Manahan-Vaughan, 2013; Wöhrl *et al*, 2007). The CA1 and SUB act as major relay stations in the hippocampus-VTA loop. The enhanced LTP at CA1-SUB synapses following systemic NMDAR antagonism is expected to result in an increased subicular output. This could lead to a positive feedback in the hippocampus-VTA loop and augmentation of the DA system. This mechanism is in line with the hippocampal hyperactivity model in which a hyperactive ventral hippocampus drives a DA hyperfunction and results in aberrant DA neuron signaling (Lodge and Grace, 2007). This might be a key factor in the development of pathological changes in dopaminergic circuits. While commonly used antipsychotics predominantly act on D2Rs, D1/D5Rs have been implicated in cognitive function (e.g. working memory). The encoding of novel information relies on the time-locked release of DA and D1/D5R activation in the hippocampus (Li *et al*, 2003). Insufficient or excessive D1/D5R stimulation seems to be deleterious to cognitive function (Lisman and Otmakhova, 2001; Williams and Castner, 2006).

Lasting hippocampus-dependent deficits after a single application of NMDAR antagonists including MK-801 that persisted for up to months have been reported (Lukoyanov and Paula-Barbosa, 2000; Manahan-Vaughan *et al*, 2008; Wieschollеck and Manahan-Vaughan, 2013; Wozniak *et al*, 1996). The enhanced subicular LTP occurred one hour after MK-801-treatment and lasted for about one week. This time-locked correlation suggests that the observed subicular plasticity could contribute to the MK-801-induced hippocampus-dependent behavioral deficits in the drug-withdrawn state. However, adaptive coping mechanisms including a compensatory upregulation of LTCC function after temporary NMDAR-antagonism have to be

considered as well. Besides that, the enhanced LTP could be attributed to the reported potent antidepressant effects of acute NMDAR antagonist administration (for a review see Browne and Lucki, 2013). Using GluN2B-selective antagonists which display antidepressant but lack the psychotomimetic effects might help to clarify this issue.

In summary, the present study is the first to reveal a region-specific presynaptic form of LTP in the hippocampus that is induced by activation of D1/D5Rs and modulated by LTCCs and NMDAR after drug-induced psychosis. Thereby, our findings may provide a cellular mechanism how NMDAR antagonism can lead to an enhanced hippocampal output causing activation of the hippocampus-VTA-loop and overdrive of the dopamine system.

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Figure Legends

Fig. 1: Systemic MK-801 application leads to subicular late-onset LTP. (a-c) LTP in CA1 following strong HFS (4x 100 pulses at 100 Hz) is significantly impaired in MK-801-treated rats (control: 100 Hz *n*=8, 40 Hz *n*=7, MK-801: 100 Hz *n*=8, 40 Hz *n*=7, nonpaired *t*-test, ***p*<0.01). **(d-f)** Attenuated HFS (10 pulses at 40 Hz) induces LTP at CA1-SUB synapses selectively in MK-801-treated rats (control: 100 Hz *n*=11, 40 Hz *n*=10, MK-801: 100 Hz *n*=9, 40 Hz *n*=9, non-paired *t*-test, ****p*<0.001). (a-c, d-f) Scale bars of EPSPs: 5 mV, 25 ms. (c+f) Columns represent mean±SEM after electrical stimulation protocol. See also Fig. S1, Table S1.

Fig. 2: LTP in MK-801 treated rats is induced by activation of D1/D5R. (a) Application of the NMDAR antagonist AP5 reveals an initial NMDAR-dependent component (min 10-15) but fails to block LTP (*n*=7). **(b)** The D2R antagonist sulpiride (*n*=9) does not block the induction of LTP in MK-801-treated rats. **(c)** Blocking D1/D5R with the D1/D5R antagonist SCH23390 before electrical stimulation prevents LTP (*n*=8). **(d)** Blocking D1/D5R after LTP induction does not prevent LTP expression (*n*=7). (a-d) MK-801 data taken from Fig. 1e is replotted for comparison. **(e)** Bath-application of SCH23390 does not affect baseline EPSP amplitudes in MK-801-treated rats (*n*=6). **(f)** Normalized sample traces of EPSP responses to HFS (10 pulses at 40 Hz) in MK-801-treated rats with or without D1/D5R blockade, scale bar: 25 ms. **(g)** Short-term depression of synaptic responses during sustained activity increases when D1/D5Rs are blocked (MK-801: *n*=9, MK-801+SCH23390: *n*=8, non-paired *t*-test, ***p*<0.01, **p*<0.05). **(h)** Bath-application of the D1/D5R agonist SKF38393 enhances EPSP amplitudes at various concentrations only in MK-801-

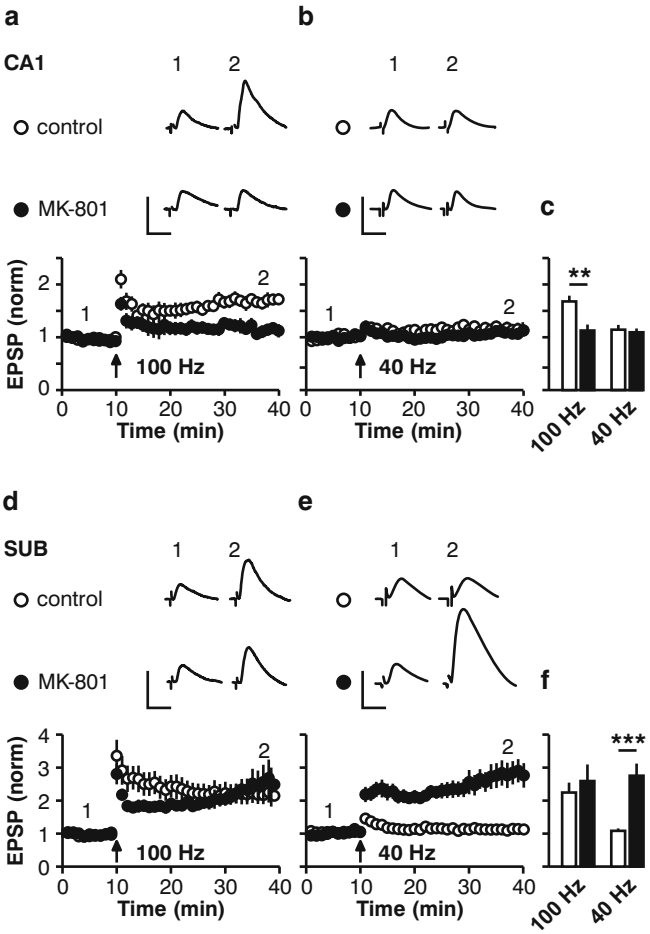
treated rats, non-paired *t*-test, 10 µM: control: *n*=6, MK-801: *n*=8 **p*<0.05, 30 µM: control: *n*=9, MK-801: *n*=7 ***p*<0.01). Scale bars of EPSPs: 2.5 mV, 25 ms. **(i)** PPI decreases after D1/D5R agonist-induced LTP in MK-801-treated rats (paired *t*-test, **p*<0.05). Scale bar of paired-pulse recordings: amplitude scaled to first pulse of SKF38393, 25 ms. Columns in (h, i) represent mean±SEM. See also Fig. S2, S3, S6.

Fig. 3: LTP in MK-801 treated rats is expressed presynaptically and is independent of postsynaptic Ca^{2+}. (a) PPI decreases after electrical induction of LTP in MK-801-treated rats (paired *t*-test, **p*<0.05). Points depict mean value of single neurons before and after LTP induction. Scale bar of paired-pulse recordings: amplitude scaled to first pulse of LTP, 25 ms. **(b)** LTP in MK-801-treated rats is associated with a decrease in failure rate of minimal stimulation, points represent single neurons (Wilcoxon test, ***p*<0.01). **(c)** Postsynaptic dialysis with the Ca^{2+} buffer BAPTA fails to prevent the expression of LTP in MK-801-treated rats (one-way ANOVA $F_{(2,20)}$=9.974, ****p*<0.001, *post-hoc* Dunnett's test: MK-801 *n*=7 ****p*<0.001, MK-801+BAPTA *n*=8 **p*<0.05, control *n*=8), scale bars of EPSCs: 100 pA, 25 ms. See also Fig. S4.

Fig. 4: Blockade of D1/D5Rs decreases mEPSC frequency at CA1-SUB synapses in MK-801-treated rats. (a) Subicular pyramidal cells are innervated by D1R-positive terminals. Confocal images of a biocytin-filled dendrite from a subicular bursting cell in control and MK-801-treated rat (i+v). D1R (ii+vi), and vGluT1 (iii+vii) immunohistochemical staining in the same area. (iv) D1R- and vGluT1-immunopositive presynaptic terminal (arrow) ending onto the distal biocytin-filled dendritic shaft seen in (i). (viii) D1R- and vGluT1-immunopositive presynaptic

terminal (arrow) terminating onto the distal biocytin-filled dendritic spine seen in (v). Scale bar: 2 µm in control and 5 µm in MK-801. **(b)** Same as in (a) but stained against D5R. (iv+viii) D5R- and vGluT1-immunopositive presynaptic terminal (arrow) terminating onto the proximal biocytin-filled dendritic spine seen in (i) and (v). Scale bar: 5 µm in (i) to (viii). **(c+d)** D1/D5R blockade reduces mEPSC frequency but not amplitude in MK-801-treated rats (paired *t*-test **p*<0.05), points represent single experiments, columns represent mean±SEM, scale bars: 10 pA, 250 ms. **(e+f)** Cumulative histograms of inter-event intervals (MK-801: Kolmogorov-Smirnov test **p*<0.05).

Fig. 5: LTP in MK-801-treated rats is mediated by the AC-cAMP-PKA cascade and modulated by LTCCs. (a) Preincubation with a PKA inhibitor prevents LTP (*n*=6). **(b)** Activation of AC enhances subicular EPSC amplitudes in both animal groups (control: *n*=6, MK-801: *n*=6), scale bars: 100 pA, 25 ms. **(c+d)** Blocking LTCCs before (*n*=9) or after (*n*=9) LTP induction impairs LTP expression. (a, c, d,) MK-801 data taken from Fig. 1e is replotted for comparison. **(e)** D1/D5R activation leads to an increased stimulus-induced Ca^{2+} fluorescence signal in MK-801-treated rats in absence of LTCC blockage (paired *t*-test, **p*<0.05), columns represent mean±SEM, scale bars of sample fluorescence recordings: 5% $\Delta F/F_0$, 1 s, arrows indicate start of stimulus (20 pulses at 20 Hz). **(f)** Summary of changes in synaptic strength following attenuated HFS (10 pulses at 40 Hz) for the different experimental groups (Kruskal-Wallis H-test ($H_{(9)}$=53.35, *p*<0.0001) followed by *post-hoc* Dunn's test). Control and MK-801 data taken from Fig. 1f is replotted for comparison. Columns represent mean±SEM after electrial stimulation protocol. See also Fig. S5, S7.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b
CA1
1
2
control
MK-801
c
EPSP (norm)
100 Hz
40 Hz
Time (min)
**
100 Hz
40 Hz
d
e
SUB
f
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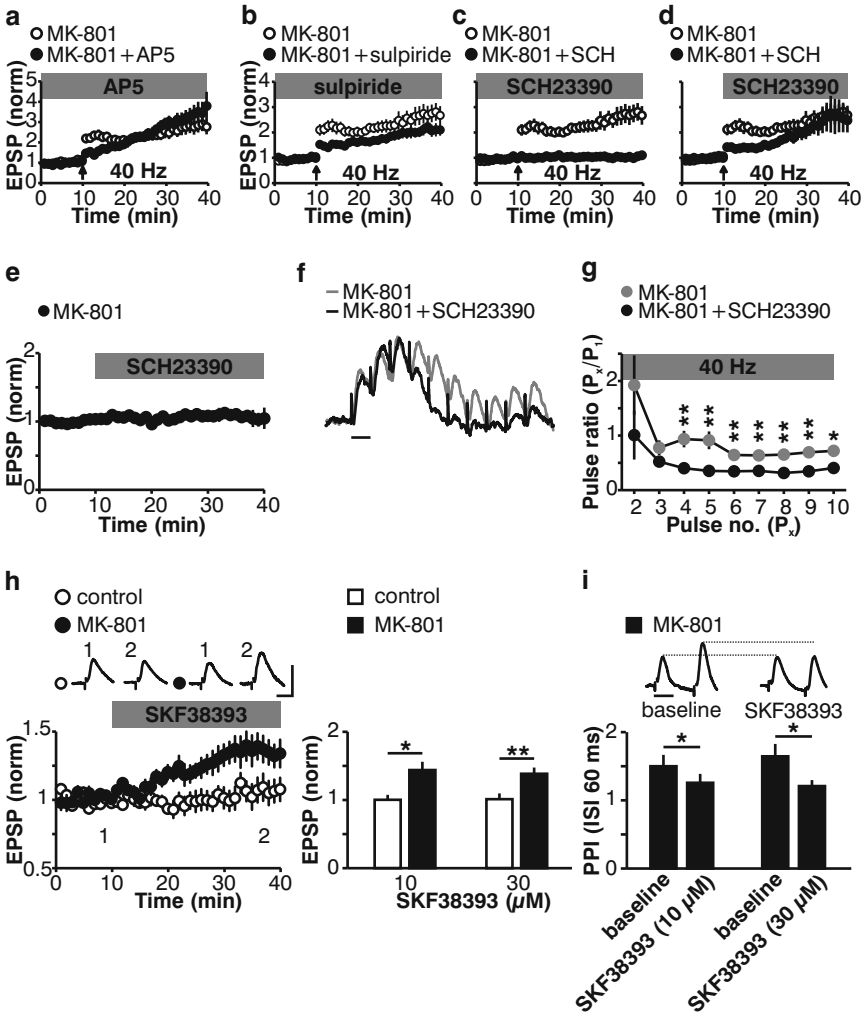
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MK-801
MK-801+AP5
AP5
40 Hz
EPSP (norm)
Time (min)
b
MK-801
MK-801+sulpiride
sulpiride
40 Hz
EPSP (norm)
Time (min)
c
MK-801
MK-801+SCH
SCH23390
40 Hz
Time (min)
d
MK-801
MK-801+SCH
SCH23390
40 Hz
Time (min)
e
MK-801
SCH23390
EPSP (norm)
Time (min)
f
MK-801
MK-801+SCH23390
g
MK-801
MK-801+SCH23390
40 Hz
Pulse ratio (Px/P1)
Pulse no. (Px)
h
control
MK-801
SKF38393
EPSP (norm)
Time (min)
control
MK-801
EPSP (norm)
SKF38393 (μM)
i
MK-801
baseline
SKF38393
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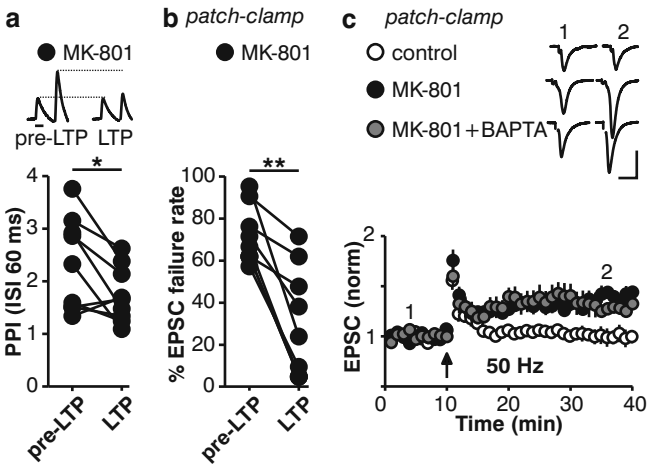
baseline
SKF38393 (10 μM)
baseline
SKF38393 (30 μM)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pre-LTP
LTP
*
PPI (ISI 60 ms)
4
3
2
1
0
pre-LTP
LTP
b patch-clamp
MK-801
**
% EPSC failure rate
100
80
60
40
20
0
pre-LTP
LTP
c patch-clamp
control
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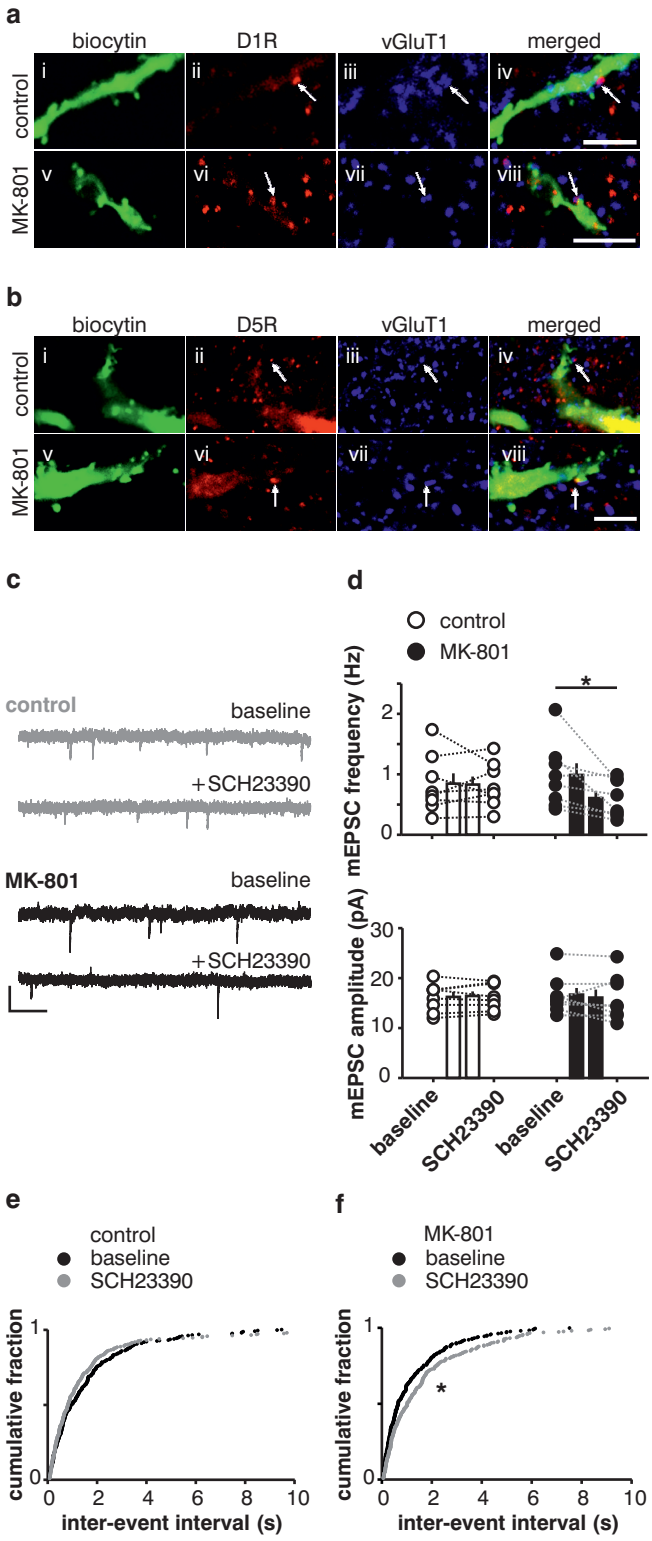
1
2
EPSC (norm)
2
1
50 Hz
0
10
20
30
40
Time (min)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iii
iv
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vi
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D5R
vGluT1
merged
control
MK-801
c
control
baseline
+SCH23390
MK-801
baseline
+SCH23390
d
control
MK-801
mEPSC frequency (Hz)
mEPSC amplitude (pA)
baseline
SCH23390
baseline
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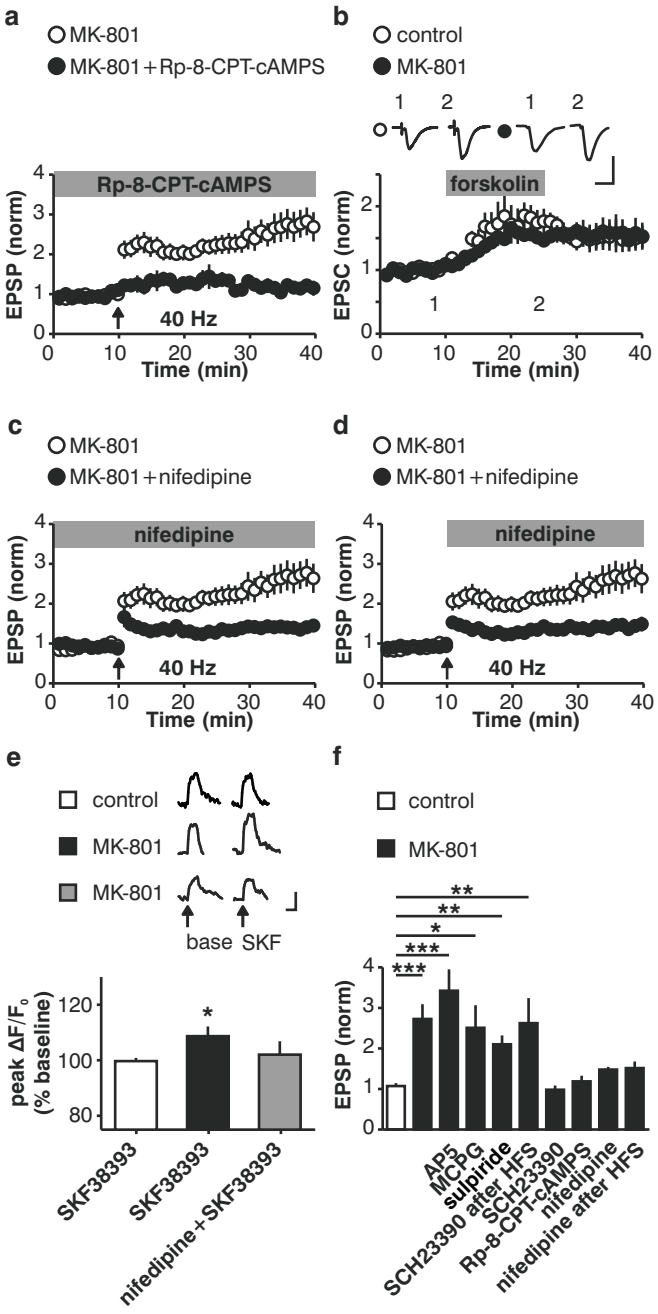
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Rp-8-CPT-cAMPS
EPSP (norm)
40 Hz
Time (min)
b
control
MK-801
1 2 1 2
forskolin
EPSC (norm)
1 2
Time (min)
c
MK-801
MK-801+nifedipine
nifedipine
EPSP (norm)
40 Hz
Time (min)
d
MK-801
MK-801+nifedipine
nifedipine
EPSP (norm)
40 Hz
Time (min)
e
control
MK-801
MK-801
base SKF
peak ΔF/F0 (% baseline)
SKF38393
SKF38393
nifedipine+SKF38393
f
control
MK-801
EPSP (norm)
AP5
MCPG
sulpiride
SCH23390 after HFS
SCH23390
Rp-8-CPT-cAMPS
nifedipine
nifedipine after HFS